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Research Article
Influence of Silver Nitrate on Enhancing *in vitro* Rooting of *Gardenia jasminoides* Ellis

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Abstract

Background and Objectives: Silver ion (Ag+), which added *in vitro* in the form of silver nitrate ($AgNO_3$) or as silver thiosulfate (STS) complex can act as an effective inhibitor of ethylene action. In addition to inhibiting ethylene action, Ag+promotes indole acetic acid (IAA). The current study was conducted to evaluate the impact of $AgNo_3$ on improvement root formation during micropropagation of *Gardenia jasminoides* Ellis. **Materials and Methods:** For multiplication, stem segments were cultured on MS medium supplemented with 0, 3, 5 or 7 mg L^{-1} benzyl adenine (BA). Elongated shootlets were transferred to different rooting treatments; MS supplemented with naphthalene acetic acid (NAA) or indole butyric acid (IBA) in combination with different concentrations of $AgNo_3$ 1, 2 and 3 mg L^{-1}. The antioxidant activity of extracts from each rooting treatment was determined through 2,2-diphenyl-1-picrylhydrazyl(DPPH) radical scavenging activity. **Results:** Using high concentration of BA (7 mg L^{-1}) led to obtain the highest multiplication rate. Results observed that addition of $AgNo_3$ to medium contained NAA alone doubled the rooting formation percentage. Meanwhile, addition of $AgNo_3$ to MS medium contained IBA alone or both NAA and IBA increased the root formation percentage from 83.33-100%. DPPH radical scavenging activity of different rooting treatments revealed that 2 NAA+2 IBA+2 $AgNo_3$ recorded the highest DPPH percentage (79. 2%). **Conclusion:** It could be concluded that, the medium supplemented with2 NAA+2 IBA gave the highest number of roots/plantlet. While, the addition of 3 mg L^{-1} $AgNo_3$ to MS medium supplemented with 2 NAA+2 IBA gave the highest mean length of root/plantlet. Also, it could be concluded that using $AgNo_3$ with 2 mg L^{-1} NAA+2 mg L^{-1} IBAA improved the antioxidant capacity of gardenia extracts.

Key words: *Gardenia jasminoides* Ellis, silver nitrate, rooting, antioxidant capacity, DPPH radical scavenging activity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Gardenia jasminoides Ellis is an evergreen ornamental plant belongs to the genus *Gardenia*, family Rubiaceae, widely grown in the tropics, subtropics and warm temperate regions. It is used as a cut flower and a garden shrub as screens, hedges or borders. Gardenia has thick, glossy, dark-green leaves and white flowers with sweet fragrance. Gardenia is propagated by terminal shoot cuttings. However, it takes several years to obtain enough plants by cuttings. *In vitro* propagation of *Gardenia* plants was a promising tool to overcome this problem[1-3]. Micropropagation became a reliable and routine approach for large-scale rapid plant multiplication. Rooting stage may involve not only rooting of shoots derived *in vitro*, but also conditioning of the *in vitro* plants to increase their potential for *ex vitro* acclimatization and survival during transplanting process[4].

Incubation of plant tissue cultures resulted in ethylene accumulation in the culture vessels, which is unfavorable for culture growth[5].

Silver ion (Ag+), which added *in vitro* in the form of silver nitrate ($AgNO_3$) or as silver thiosulfate (STS) complex can act as an effective inhibitor of ethylene action[6]. As well as, it was found that in addition to inhibiting ethylene action, Ag^+ promotes[7] IAA. Thus, silver supplementation to the culture medium has been used to improve plant regeneration *in vitro*[8,9]. In this regard, silver nitrate is widely used in plant tissue culture. Many reports have shown the positive effect of $AgNO_3$ on shoot multiplication, root formation and flowering *in vitro*[10-16].

DPPH radical scavenging assay is a good method depend on the antioxidant efficiency of plant crude extracts, based on the conversion of the free radical 2,2-diphenyl-1-picrylhydrazyl to a stable molecule diphenylpicryl hydrazine by reduction using various plant extracts as hydrogen donors, through change the color from purple to yellow in a short time[17]. The aim of the current study is to evaluate the impact of silver nitrate on improvement root formation during micro propagation of *Gardenia jasminoides* Ellis. and its effect on the antioxidant efficiency of plant crude extracts through DPPH radical scavenging assay.

MATERIALS AND METHODS

This study was carried out in Plant Biotechnology Department, National Research Centre, Giza, Egypt, during the period from January-December, 2017.

Plant material: *In vitro* growing *Gardenia* plant lets were subjected in this study as a source of plant material.

Multiplication stage: Stem segments (about 1 cm length) were used as explants. For multiplication explants were cultured on MS medium supplemented with 0 (control), 3, 5 or 7 mg L^{-1} BA, for two subculture, each of them was 4 weeks. Mean number of lateral buds/explant and mean numbers of lateral shoots were recorded after the two subcultures.

Elongation stage: Cultures from the previous stage were transferred to MS medium supplemented with 2 mg L^{-1} BA or 0.4 mg L^{-1} IAA as well as interaction between 1 mg L^{-1} BA+0.2 mg L^{-1} IAA as an elongation medium for two subcultures each of them was 4 weeks. Average number of lateral shoots, shoot height and number of leaves per shoot were recorded after the two subcultures.

Rooting stage: The elongated shoots (about 3 cm) were transferred to different rooting medium supplemented with different concentrations of NAA and IBA as well as silver nitrate as follow:

- Treatment
- 3 mg L^{-1} NAA
- 3 mg L^{-1} NAA+1 mg L^{-1} $AgNo_3$
- 3 mg L^{-1} NAA+2 mg L^{-1} $AgNo_3$
- 3 mg L^{-1} NAA+3 mg L^{-1} $AgNo_3$
- 3 mg L^{-1} IBA
- 3 mg L^{-1} IBA+1 mg L^{-1} $AgNo_3$
- 3 mg L^{-1} IBA+2 mg L^{-1} $AgNo_3$
- 3 mg L^{-1} IBA+3 mg L^{-1} $AgNo_3$
- 2 mg L^{-1} NAA+2 mg L^{-1} IBA
- 2 mg L^{-1} NAA+2 mg L^{-1} IBA+1 mg L^{-1} $AgNo_3$
- 2 mg L^{-1} NAA+2 mg L^{-1} IBA+2 mg L^{-1} $AgNo_3$
- 2 mg L^{-1} NAA+2 mg L^{-1} IBA+3 mg L^{-1} $AgNo_3$

At the end of the second subculture, rooting formation percentage, number of roots/plantlet, length of roots (cm)/plantlet and plantlet height (cm) were recorded.

Biochemical analysis

Sample extraction: According to Gabr *et al.*[18], 50 mg dry weight (from all rooting treatments which were air dried at room temperature for 3 days) was grounded and extracted with methanol 80% (1 mL) for 48 h on a shaker (120 rpm) at room temperature. The extraction procedure was

carried out in an ultrasonic water bath for 20 min. Samples were centrifuged for 5 min at 6000 rpm. Then the supernatants were collected. The extracts were stored at -20°C until further use.

DPPH radical scavenging activity: The DPPH assay according to Gabr *et al.*[18] was used with some modifications. Methanolic extract of different treatments of rooting (0.1 mL of each) were vortexed for 30 sec with1.9 mL of DPPH solution and left to react for 30 min, after which the absorbance at 515 nm was recorded. A control with no added extract was also analyzed. Scavenging activity was calculated as follow:

$$\text{DPPH radical-scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where, A is the absorbance at 515 nm.

Statistical analysis: All analysis was in triplicate and data reported as Mean±Standard Deviation (SD). Data were subjected to analysis of variance (One-way ANOVA) ($p<0.05$). Results were processed by Excel (Microsoft office 2010) and SPSS version 17.0 (SPSS inc, Chicago, IL, USA).

RESULTS AND DISCUSSION

Multiplication stage: The effect of different concentrations of BA on number of lateral buds and lateral shoots was recorded in Table 1.

Data generally showed a remarkable effect of the different BA concentrations on mean number of lateral bud/explants and number of lateral shoots compared with control treatment (without BA).

Using high concentration of BA (7 mg L^{-1}) led to obtain the highest mean number of shoots per explant (2.33). On the other hand, the lowest mean number of shoot per explant (0.66 shoot/explant) was observed with 3 mg L^{-1} BA. The high concentrations of BA (5 or 7 mg L^{-1}) produce swelling lateral buds after 4 weeks of culturing which, developed to very short shoots as a cluster shape after 8 weeks of culturing. These results are in agreement with those reported by Chuenboonngarm *et al.*[19] on *Gardenia jasminoides* who found that using B5 medium supplemented with BA gave high shoots number compared with those cultured on a medium free from BA. On the same respect, Duhoky and Rasheed[20] reported that using a medium supplemented with 2 mg L^{-1} BA gave the highest shoot number/each explant (2.20 shoots/explant), which may be related to the efficiency of BA in cell division. Furthermore, in *Gardenia gummifera*, multiple shoots were obtained from nodal explants on MS medium fortified[21] with 2 mg L^{-1} BA+0.5 mg L^{-1} IBA. While, MS medium supplemented with 1 mg L^{-1} BA+0.5 mg L^{-1} IBA gave the highest number of shoots from leaf and internode explants of *Gardenia gummifera*[22]. In this connection, Lakshmi and Jaganmohanreddy[14] reported that, using inter-nodal segments explants of *Gardenia resinifera* Roth, the medium supplemented with 2 mg L^{-1} BA+0.25 mg L^{-1} NAA gave two shoots with an average shoot length 3.75 cm. As well as, using a medium fortified with 2 mg L^{-1} BA+0.1 mg L^{-1} IAA gave two shoots with average shoot length 3.53 cm.

Elongation stage: The new shoots obtained in the previous stage were transferred to culture medium supplemented with BA (2 mg L^{-1}) or IAA (0.4 mg L^{-1}) or 1 mg L^{-1} BA+0.2 mg L^{-1} IAA aiming to prolongate the shoots. Data presented in Table 2 showed that highest mean number of lateral shoots (4 shoots) was obtained with 2 mg L^{-1} BA whereas, the lowest mean number of lateral shoots (2.5 shoot) was recorded with 0.4 mg L^{-1} IAA. On the other hand, the treatment of 1 mg L^{-1} BA+0.2 mg L^{-1} IAA gave the highest mean number of leaves (8.5 leaves/shoot). However, the highest of the main shoot height (3.33 cm) was recorded with 2 mg L^{-1} BA. These results were partially in agreement with those found with Duhoky and Rasheed[23], who reported that using MS medium supplemented with 3 mg L^{-1}/BA+0.9 mg L^{-1}/1 IAA with single nodes of gardenia gave the highest number of shoots and leaves. Also, Salim and Hamza[24] reported that using a medium

Table 1: Effect of different concentrations of BA on number of lateral buds and number of lateral shoots after 8 weeks of culturing

Benzyl adenine (mg L^{-1})	Mean no. of lateral buds/explant	Mean no. of lateral shoots/explant
0	-	-
3	1.0±1.67	0.66±1.03
5	1.5±1.22	0.83±1.32
7	2.0±1.26	2.33±2.06

Table 2: Effect of BA, IAA and their interaction added to MS-medium on number of lateral shoots, number of leaves per shoot and shoot height, after 8 weeks of culturing

Treatments	Mean number of lateral shoots	Mean number of leaves/shoot	Shoot height (cm)
2 mg L^{-1} BA	4.00±2.09	6.66±4.13	3.33±1.75
0.4 mg L^{-1} IAA	2.50±1.51	6.66±1.03	2.16±0.98
1 mg L^{-1} BA+0.2 mg L^{-1} IAA	2.66±2.87	8.50±2.81	2.33±1.12

Mean±SD

Table 3: Effect of NAA, IBA and $AgNo_3$ on root formation, mean number of roots/plant and mean length of root/plantlet

Treatment (mg L^{-1})	Rooting formation (%)	Mean of number of roots/plantlet	Mean of length of roots (cm)/plantlet	Mean plantlet height (cm)
3 NAA	33.33	8.00±1.73	1.166±0.76	4.00±1.0
3 NAA+1 $AgNo_3$	66.66	8.33±3.21	1.06±0.15	2.33±1.04
3 NAA+2 $AgNo_3$	66.66	8.33±3.21	1.00±0.15	2.83±0.28
3 NAA+3 $AgNo_3$	66.66	11.6±2.08	1.06±0.40	5.16±1.89
3 IBA	83.33	13.00±1.73	1.10±0.36	5.00±0.0
3 IBA+1 $AgNo_3$	100.00	16.33±3.51	0.70±0.36	4.66±0.76
3 IBA+2 $AgNo_3$	100.00	11.66±1.52	0.33±1.52	4.16±0.76
3 IBA+3 $AgNo_3$	100.00	14.00±2.64	1.13±0.58	6.66±1.52
2 NAA+2 IBA	83.33	20.66±4.04	0.83±0.30	5.66±1.15
2 NAA+2 IBA+1 $AgNo_3$	100.00	12.33±2.51	1.43±0.60	4.5±1.73
2 NAA+2 IBA+2 $AgNo_3$	100.00	15.66±3.21	1.20±0.3	5.5±1.73
2 NAA+2 IBA+3 $AgNo_3$	100.00	13.00±4.00	2.66±0.28	5.5±0.50

Mean±SD

supplemented with 3.0 mg L^{-1} TDZ+0.3 mg L^{-1} IAA with *Gardenia jasmonides* gave the best results for shoot multiplication as shoot number, shoot length and number of leaves/shoot.

It is well known that auxins (IAA) is involved in root initiation. Apical dominance may occur in which the growth of lateral buds is inhibited by the growth of apical buds and that declared that in case of adding IAA alone to the elongation medium gave the lowest number of lateral shoots. Whereas, cytokinins (BA) is produced in the regions where cell division occurs; i.e., in the roots and shoots. They help in overcoming apical dominance, leaves production and growth of lateral shoots. This may explain why the addition of IAA in combination with BA gave the highest mean number of leaves and also, the highest of the main shoot height was recorded with 2 mg L^{-1} BA.

Rooting stage: One of the major obstacles of the *in vitro* propagation is the rooting and conditioning of the plantlets. The elongated shoots were transferred to MS-medium contained two concentrations (2 and 3 mg L^{-1}) of NAA or IBA added individually or in combination and supplemented with $AgNo_3$ as reported in Table 3. Root formation percentage, number of roots/plantlet, root length and plantlet height was recorded after 8 weeks of culturing. The results obtained indicated that the highest root formation percentage (100%) were recorded with IBA at 3 mg L^{-1} with silver nitrate at any concentration (1, 2, 3 mg L^{-1}) and with 2 mg L^{-1} NAA+2 mg L^{-1} IBA in the presence of silver nitrate at any concentration.

Also, it can be noticed that addition of $AgNo_3$ at one of the three concentrations (1, 2 or 3 mg L^{-1}) to 3 mg L^{-1} NAA doubled the rooting formation percentage (from 33-66%). Meanwhile, addition of $AgNo_3$ to MS medium contained IBA alone or both NAA and IBA increased the root formation percentage from 83.33-100%. Also, it was observed that the highest mean number of roots/plantlet was 20.66 roots/plantlet on medium supplemented with 2 mg L^{-1} NAA+2 mg L^{-1} IBA (Fig. 1i) followed by 16.3 roots/plantlet with medium supplemented with 3 mg L^{-1} IBA+1 mg L^{-1} $AgNo_3$ (Fig. 1f).

On the other hand, it was found that addition of 3 mg L^{-1} $AgNo_3$ to both NAA and IBA at 2 mg L^{-1} gave the highest mean of length of roots/plantlet (2.66 cm) (Fig. 1l).

Regarding plantlet height, results presented in Table 3 revealed that, the highest plantlet height (6.66 cm) was obtained on medium supplemented with 3 IBA+3 $AgNo_3$ (Fig. 1h).

It was noticed that thickness of roots was affected by the type of the auxins as generally roots were thick with NAA alone or with $AgNo_3$ whereas it were thin with IBA alone or with $AgNo_3$.

Incubation of plant tissue cultures *in vitro* resulted in ethylene accumulation in the culture vessels, which is unfavorable for culture growth[5]. Silver ion (Ag+), which added *in vitro* in the form of silver nitrate ($AgNO_3$) or as silver thiosulfate (STS) complex can act as an effective inhibitor of ethylene action[6,25]. In this regard, Strader *et al.*[7] reported that in addition to inhibiting ethylene action, Ag^+ promotes IAA. Thus, silver supplementation to the medium has been used to improve plant regeneration *in vitro*[8,9].

The results assured that silver nitrate could be the solution of slow multiplication and difficult *in vitro* root formation problems. These results were in harmony with Harsh *et al.*[26], who reported that in case of adding 0.5 mg L^{-1} IAA to the rooting medium of *Decalepis hamiltonii* poor rooting was observed. The resultant roots were stunted. While, addition of 40 μM $AgNO_3$ improved rooting as root initiation and root elongation. Similar results were conducted in *Vanilla planifolia* as $AgNo_3$ not only induced shoot multiplication but also influenced rooting[11].

Fig. 1(a-l): *Gardenia* plantlet after 8 weeks on rooting medium supplemented with, (a) 3 mg L^{-1} NAA, (b) 3 mg L^{-1} NAA+ 1 mg L^{-1} $AgNo_3$, (c) 3 mg L^{-1} NAA+2 mg L^{-1} $AgNo_3$, (d) 3 mg L^{-1} NAA+3 mg L^{-1} $AgNo_3$, (e) 3 mg L^{-1} IBA, (f) 3 mg L^{-1} IBA+1 mg L^{-1} $AgNo_3$, (g) 3 mg L^{-1} IBA+2 mg L^{-1} $AgNo_3$, (h) 3 mg L^{-1} IBA+3 mg L^{-1} $AgNo_3$, (i) 2 mg L^{-1} NAA+2 mg L^{-1} IBA, (j) 2 mg L^{-1} NAA+2 mg L^{-1} IBA+1 mg L^{-1} $AgNo_3$, (k) mg L^{-1} NAA+2 mg L^{-1} IBA+2 mg L^{-1} $AgNo_3$and (l) 2 mg L^{-1} NAA+2 mg L^{-1} IBA+3 mg L^{-1} $AgNo_3$

Also, Chithra *et al.*[27] reported that silver nitrate induce *in vitro* rooting on *Rotula aquatica* L. They added that the addition of 11.7 µM silver nitrate to half strength MS liquid medium containing 2.69 µM NAA increased the number of roots to reach 16.8/explant comparing with 7.3 roots/shoot without the addition of silver nitrate. Also, Petrova *et al.*[13] reported that using half strength MS supplemented with 1 mg L^{-1} IBA+1 mg L^{-1} $AgNo_3$ with *Gentiana lutea* gave 90% rooting with 4.5 roots/plant with 1.5 cm mean length. Mahmoud and Kosar[28] reported that the highest elongation and number of roots per strawberry explant were obtained on MS medium supplemented with

Table 4: Effect of NAA, IBA and $AgNo_3$ on free radical scavenging capacity of DPPH (%) for gardenia extracts

Treatments	DPPH (%)
3 NAA	62.4±0.002
3 NAA+1 $AgNo_3$	61.6±0.004
3 NAA+2 $AgNo_3$	66.4±0.107
3 NAA+3 $AgNo_3$	77.2±0.009
3 IBA	64.0±0.001
3 IBA+1 $AgNo_3$	58.4±0.012
3 IBA+2 $AgNo_3$	49.6±0.031
3 IBA+3 $AgNo_3$	63.2±0.003
2 NAA+2 IBA	55.6±0.111
2 NAA+2 IBA+1 $AgNo_3$	62.4±0.025
2 NAA+2 IBA+2 $AgNo_3$	79.2±0.007
2 NAA+2 IBA+3 $AgNo_3$	62.8±0.009

Mean of DPPH percentage±SD

0.5 mg L^{-1} BAP+0.2 mg L^{-1} KIN+4 mg L^{-1} Ag(NO). Tamimi[15] on his study on banana declared that the rooting media fortified with10 mg L^{-1} $AgNO_3$ gave the highest number of roots per shoot (21.7) and the longest roots (12.68 cm). Geetha *et al.*[16] in their study on *Solanum nigrum* reported that, 95% of rooting, highest number of roots (24.6±0.26) were obtained with MS medium fortified with 2.0 mg L^{-1} IBA and 0.4 mg L^{-1} $AgNO_3$ and highest mean root length (6.5±0.36 cm) was observed on the same medium. As for *Gardenia*, Duhoky and Rasheed[20] reported that the medium supplemented with 8 mg L^{-1} NAA gave the highest percentage of rooting (90%) and the medium fortified by 12 mg L^{-1} IAA gave (100%) rooting of *Gardenia jasmonides*. However, Gajakosh *et al.*[29] mentioned that using 5.0 mg L^{-1} IBA and 5.0 mg L^{-1} IAA was suitable for induction of roots in *Gardenia gummifera*.

On the other hand, Lakshmi and Jaganmohanreddy[14] stated that regenerated shoots of *Gardenia resinifera* roth were rooted on 1/2 MS supplemented with IAA (0.5 mg L^{-1}).

It could be concluded that, the medium supplemented with2 NAA+2 IBA gave the highest number of roots/plantlet (Fig. 1i). While, the addition of 3 mg L^{-1} $AgNo_3$ to MS medium supplemented with 2 NAA+2 IBA gave the highest mean length of root/plantlet (Fig. 1l). While, the medium supplemented with 3 mg L^{-1} IBA+3 mg L^{-1} $AgNo_3$ gave the highest mean height of plantlet (Fig. 1h).

DPPH radical scavenging assay for gardenia extracts: The DPPH percentage was recorded Table 4. Using 2 NAA+2 IBA+2 $AgNo_3$ recorded the highest DPPH percentage (79. 2%) following by using 3 NAA+3 $AgNo_3$ (77.2%). While using 3 IBA+2$AgNo_3$ recorded the lowest DPPH percentage (49.6%). When using 3 mg L^{-1} NAA or 2 mg L^{-1} NAA+2 mg L^{-1} IBA the DPPH percentage increased with adding different concentrations of $AgNo_3$. While, using 3 mg L^{-1} IBA with different concentrations of $AgNo_3$ decreased the DPPH percentage.

From these results it could be concluded that using $AgNo_3$ with 3 mg L^{-1} NAA or 2 mg L^{-1} NAA+2 mg L^{-1} IBAA increased the efficiency of the extracts for donating hydrogen atom to improve their antioxidant capacity. While, using $AgNo_3$ with 3 mg L^{-1} IBA decreased this efficiency.

The current results were in line with those found by Sahandi *et al.*[30], where the content of polyphenols in vegetal tissues of borage showed an increase proportional to the concentration of silver nitrate in the range of 100-300 mg L^{-1}. Juarez-Maldonado *et al.*[31] stated that silver nitrate had a positive effect on total antioxidant capacity of onion bulbs when it was applied as a nutritive solution. But it differs from those found by De la Fuentei *et al.*[32], who stated that watermelon plants obtained 30 mg L^{-1} silver nitrate showed the greatest total amount of antioxidants in fruits comparing with those obtained higher concentration of silver nitrate.

CONCLUSION

It could be concluded that, the medium supplemented with2 NAA+2 IBA gave the highest number of roots/plantlet. While, the addition of 3 mg L^{-1} $AgNo_3$ to MS medium supplemented with 2 NAA+2 IBA gave the highest mean length of root/plantlet. Also, it could be concluded that using $AgNo_3$ with 2 mg L^{-1} NAA+2 mg L^{-1} IBAA increased the efficiency of the extracts for donating hydrogen atom to improve their antioxidant capacity.

SIGNIFICANCE STATEMENT

Actually, the main aim of this paper is to study the effect of silver nitrate, as an antioxidant compound as well as promoting rooting of *in vitro* culture, on gardenia and its effect on the internal antioxidant activity of *Gardenia*. The results of this study indicate that silver nitrate could be the solution difficult *in vitro* root formation problems and enhancing the antioxidant activity of *Gardenia*.

REFERENCES

1. Dumanois, C., B. Godin and C. Bigot, 1984. *In vitro* vegetative multiplication of *Gardenia-jasminoides* Ellis. J. Plant Physiol., 116: 389-407.

2. Economou, A.S. and M.J. Spanoudaki, 1985. *In vitro* propagation of gardenia. HortScience, 200: 213-213.
3. Economou, A.S. and M.J. Spanoudaki, 1986. The influence of cytokinins and gibberellic acid on *gardenia* tissue cultures. Sci. Hortic., 29: 155-161.
4. Gajdosova, A., M.G. Ostrolucka, G. Libiakova and E. Ondruskova, 2007. Protocol for Micropropagation of *Vaccinium Vitis-Idaea* L. In: Protocols for Micropropagation of Woody Trees and Fruits, Jain, S.M. and H. Haggman (Eds.). Springer, New York, pp: 447-464.
5. Steinitz, B., N. Barr, Y. Tabib, Y. Vaknin and N. Bernstein, 2010. Control of *in vitro* rooting and plant development in *Corymbia maculata* by silver nitrate, silver thiosulfate and thiosulfate ion. Plant Cell Rep., 29: 1315-1323.
6. Beyer, E.M., 1979. Effect of silver ion, carbon dioxide and oxygen on ethylene action and metabolism. Plant Physiol., 63: 169-173.
7. Strader, L.C., E.R. Beisner and B. Bartel, 2009. Silver ions increase auxin efflux independently of effects on ethylene response. Plant Cell., 21: 3585-3590.
8. Kumar, P.P., P. Lakshmanan and T.A. Thorpe, 1998. Regulation of morphogenesis in plant tissue culture by ethylene. *In vitro* Cell. Dev. Biol. Plant., 34: 94-103.
9. Kumar, V., A. Ramakrishna and G.A. Ravishankar, 2007. Influence of Different Ethylene Inhibitors on Somatic Embryogenesis and Secondary Embryogenesis from *Coffea canephora* P ex Fr. *In vitro* Cell. Dev. Biol. Plant, 43: 602-607.
10. Bais, H.P., G.S. Sudha and G.A. Ravishankar, 2000. Putrescine and silver nitrate influences shoot multiplication, *in vitro* flowering and endogenous titers of polyamines in *Cichoorium intybus* L. cv. Lucknow local. J. Plant Growth Regul., 19: 238-248.
11. Giridhar, P., B.O. Reddy and G.A. Ravishankar, 2001. Silver nitrate influences *in vitro* shoot multiplication and root formation in *Vanilla planifolia* Andr. Curr. Sci., 81: 1166-1170.
12. Reddy, B.O., P. Giridhar and G.A. Ravishankar, 2001. *In vitro* rooting of *Decalepis hamiltonii* Wight & Arn., an endangered shrub, by auxins and root-promoting agents. Curr. Sci., 81: 1479-1482.
13. Petrova, M., E. Zayova and A. Vitkova, 2011. Effect of silver nitrate on *in vitro* root formation of *Gentiana lutea*. Roman. Biotechnol. Lett., 16: 53-58.
14. Lakshmi, B.J. and K. Jaganmohanreddy, 2013. *In vitro* multiple shoot induction directly from different explants of *Gardenia resinifera* Roth. Biosci. Biotechnol. Res. Asia, 10: 275-281.
15. Tamimi, S.M., 2015. Effects of ethylene inhibitors, silver nitrate ($AgNO_3$), cobalt chloride ($CoCl_2$) and aminooxyacetic acid (AOA), on *in vitro* shoot induction and rooting of banana (*Musa acuminata* L.). Afr. J. Biotechnol., 14: 2511-2516.
16. Geetha, G., K. Harathi and C.V. Naidu, 2016. Role of silver nitrate on *in vitro* flowering and shoot regeneration of *Solanum nigrum* (L.)—An important multipurpose medicinal plant. Am. J. Plant Sci., 7: 1021-1032.
17. Katalinic, V., M. Milos, T. Kulisic and M. Jukic, 2006. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem., 94: 550-557.
18. Gabr, A.M.M., N.M. Arafa, A.A.E.L. El-Ashry and M.K. El-Bahr, 2017. Impact of zeatin and thidiazuron on phenols and flavonoids accumulation in callus cultures of gardenia (*Gardenia jasminoides*). Pak. J. Biol. Sci., 20: 328-335.
19. Chuenboonngarm, N., S. Charoonsote and S. Bhamarapravati, 2001. Effect of BA and 2iP on shoot proliferation and somaclonal variation of *Gardenia jasminoides* Ellis *in vitro* culture. Sci. Asia, 27: 137-141.
20. Duhoky, M.M.S. and K.A. Rasheed, 2009. Micropropagation of gardenia (*Gardenia jasmonides*) by using single nodes. Mesopotamia J. Agric., Vol. 37.
21. Mir, F.A., A.S. Yadav and S. Wankhede, 2010. Efficiency of media's for propagation of medicinal tree *Gardenia gummifera* Linn. f-An endangered medicinal plant. J. Phytol., 2: 47-51.
22. Mir, F.A., A.S. Yadav, A. Bajaj, A.K. Sharma, A.K. Rai, S.A. Lone and D.K. Raghuwanshi, 2010. Organogenesis from leaf and internode explants of *Gardenia gummifera* Linn. f.-An endangered medicinal plant. Asian J. Exp. Sci., 24: 45-50.
23. Duhoky, M.M.S. and K.A. Rasheed, 2010. Effect of different concentration of BA and IAA on micropropagation of *Gardenia jasminoides*. Mesopotamia J. Agric., 38: 16-30.
24. Salim, S.A.A.R. and S.Y. Hamza, 2017. An efficient protocol for micro propagation of *Gardenia jasminoides* Ellis. Biosci. Biotechnol. Res. Asia, 14: 757-766.
25. Veen, H. and S.C. van de Geijn, 1978. Mobility and ionic form of silver as related to longevity of cut carnations. Planta, 140: 93-96.
26. Bais, H.P., G. Sudha, B. Suresh and G.A. Ravishankar, 2000. Silver nitrate influences *in vitro* root formation in *Decalepis hamiltonii* Wight & Arn. Curr. Sci., 79: 894-898.
27. Chithra, M., K.P. Martin, C. Sunandakumari and P.V. Madhusoodanan, 2004. Silver nitrate induced rooting and flowering *in vitro* on rare rhoeophytic woody medicinal plant, *Rotula aquatica* lour. Indian J. Biotechnol., 3: 418-421.
28. Mahmoud, O. and M. Kosar, 2014. *In vitro* achievement of strawberry roots formation using Ag (NO3). Am.-Eurasian J. Agric. Environ. Sci., 14: 1281-1286.

29. Gajakosh, A.M., M. Jayaraj, A.B. Sonappanavar, A.N. Bagadekar and P.V. Pattar, 2011. *In vitro* morphogenic response of leaf and shoot tip explants of *Gardenia gummifera*, (L.) f.-A medicinal plant. Int. J. Pharm. Technol., 3: 2487-2495.
30. Sahandi, M.S., A. Sorooshzadeh, H. Rezazadeh and H.A. Naghdibadi, 2011. Effect of nano silver and silver nitrate on seed yield of borage. J. Med. Plants Res., 5: 171-175.
31. Juarez-Maldonado, A., J.L. Rosales-Velazquez, H. Ortega-Ortiz, M. Cabrera-De-la-Fuente, H. Ramirez and A. Benavides-Mendoza, 2013. Accumulation of silver nanoparticles and its effect on the antioxidant capacity in *Allium cepa* L. Phyton-Int. J. Exp. Bot., 82: 91-97.
32. La Fuente, M.C.D., H. Ortega-Ortiz, A. Benavides-Mendoza and A. Sandoval-Rangel, 2014. Effect of the application of silver nitrate on antioxidant status in watermelon plants. Pak. J. Bot., 46: 1843-1846.